Plant Physiol. (1997) 113: 327–334

Observations of Ice Nucleation and Propagation in Plants Using Infrared Video Thermography[1]

Michael Wisniewski*, Steven E. Lindow, and Edward N. Ashworth

United States Department of Agriculture, Agricultural Research Service, 45 Wiltshire Road, Kearneysville, West Virginia 25430 (M.W.); Department of Plant and Microbial Biology, 151 Hilgard Hall, University of California, Berkeley, California 94720 (S.E.L.); and Department of Horticulture, Purdue University, West Lafayette, Indiana 47907 (E.N.A.)

We evaluated the use of infrared (IR) video thermography to observe directly ice nucleation and propagation in plants. An imaging radiometer with an HgCdTe long-wave (8–12 μm) detector was utilized to image the thermal response of plants during freezing. IR images were analyzed in real time and recorded on videotape. Information on the videotape was subsequently accessed and analyzed utilizing IR image analysis software. Freezing of water droplets as small as 0.5 μL was clearly detectable with the radiometer. Additionally, a comparison of temperature tracking data collected by the radiometer with data collected with thermocouples showed close correspondence. Monitoring of an array of plant species under different freezing conditions revealed that ice nucleation and propagation are readily observable by thermal imaging. In many instances, the ice nucleation-active bacterium *Pseudomonas syringae* placed on test plants could be seen to initiate freezing of the whole plant. Apparent ice nucleation by intrinsic nucleators, despite the presence of ice nucleation-active bacteria, was also evident in some species. Floral bud tissues of peach (*Prunus persica*) could be seen to supercool below the temperature of stem tissues, and ice nucleation at the site of insertion of the thermocouple was frequently observed. Rates of propagation of ice in different tissues were also easily measured by thermal imaging. This study demonstrates that IR thermography is an excellent method for studying ice nucleation and propagation in plants.

Frost-sensitive plant species have limited ability to tolerate ice formation in their tissues (Cary and Mayland, 1970; Burke et al., 1976). Although not damaged by cold temperatures alone, these species exhibit freeze damage when ice formation occurs. The control of frost injury to such species is achieved by avoiding ice formation. One way to do this is to warm the plant to temperatures above the freezing point of the tissue. Alternatively, plants can supercool to some extent below 0°C and avoid damaging ice formation (Lucas, 1954; Modlibowska, 1962; Cary and Mayland, 1970; Burke et al., 1976; Lindow et al., 1978; Marcellos and Single, 1976, 1979; Proebsting et al., 1982; Ashworth and Kieft, 1995; Lindow, 1995). The temperature to which plants can supercool varies in plant species and is influenced by the presence of ice-nucleating agents that may be of plant (Ashworth and Davis, 1984; Anderson and Ashworth, 1985; Andrews et al., 1986; Gross et al., 1988) or bacterial (King et al., 1954; Kaku, 1964; Lindow et al., 1978, 1982; Lindow, 1982, 1983, 1985, 1995; Gross et al., 1984; Hirano et al., 1985) origin.

Although the abundance of ice nuclei on plants can be estimated by freezing droplets of plant macerates or small portions of plant tissue (Lindow et al., 1982; Ashworth and Kieft, 1995), these procedures are destructive and do not provide information concerning the location of ice nucleation. Likewise, ice formation in intact plants can be readily detected by measuring the heat that is released upon the freezing of the water in the plant (Cary and Mayland, 1970; Quamme et al., 1972; Burke et al., 1976; Proebsting et al., 1982; Ashworth and Davis, 1984; Anderson and Ashworth, 1985; Ashworth et al., 1985; Andrews et al., 1986; Yelenosky 1991a, 1991b; Ashworth and Kieft, 1995). However, even when arrays of temperature-measuring devices are attached to plants, the actual site of ice initiation and the temperature at the site where ice nucleation occurred can only be inferred (Ashworth et al., 1985).

Knowledge of the relative importance of ice nucleation and inoculation from elsewhere in a plant on the freezing of a particular plant part is important in devising strategies for frost control. Such information is critical to evaluating the strategy of enhancing supercooling of plants as a means of avoiding damaging ice formation. Unfortunately, a knowledge of the processes of freezing in whole plants, particularly under realistic field conditions, is lacking. A method is needed that would reveal where ice initially forms in plants and how it propagates to explain inconsistencies observed in the control of frost damage by reducing ice nucleation-active (referred to as Ice^+) bacteria and to elucidate the role of intrinsic ice-nucleating agents in frost damage (Lindow et al., 1978, 1982, 1983; Lindow, 1982, 1985; Hirano et al., 1985; Gross et al., 1988; Proebsting and Gross, 1988).

In this study we evaluated IR video thermography under controlled conditions as a method to directly observe ice nucleation (i.e. initial ice formation) and propagation in plants as revealed by changes in temperature caused by the release of the heat of fusion as water changes phase from a liquid to a solid. Although this approach has been previ-

[1] Video sequences of the experiments documented in this study are available for educational purposes in NTSC or PAL format by sending a blank tape and cover letter (specifying NTSC or PAL format) to the corresponding author.

* Corresponding author; e-mail mwisniew@asrr.arsusda.gov; fax 1–304–728–2340.

ously used to study these processes in plants (Le Grice et al., 1993; Ceccardi et al., 1995), these preliminary evaluations were rather limited in scope, and recent advances in temperature sensitivity and accuracy, as well as spatial resolution, have greatly improved this technology. Previous attempts at using this technology rendered poor-quality images in which the details of ice nucleation and propagation were difficult to discern. In this study we addressed whether IR video thermography devices had sufficient spatial and thermal resolution to detect initial ice nucleation events and to determine patterns of subsequent ice propagation in a variety of plant species and tissue types.

MATERIALS AND METHODS

IR Imager

An imaging radiometer[2] (model 760, Inframetrics, North Billerica, MA) with an HgCdTe long-wave (8–12 μm) detector was utilized to monitor and image the thermal response of bacterial suspensions of *Pseudomonas syringae* or plants during freezing. IR images were analyzed in real time and recorded on videotape. Information could be subsequently recovered from the videotape by reviewing it with proprietary IR image analysis software (Inframetrics). Selected digital images of a video sequence were selected to highlight important details, and color negatives of these images were made for presentation. The user is allowed to select a temperature span during the operation of the camera. The intermediary temperatures of the selected span are displayed as a gray scale (or selected color palette). The midpoint of the selected temperature range can be changed up or down at the operator's discretion. Temperatures below the selected span are typically displayed as black and those above the upper limit of the temperature range are displayed as white. In most cases, a temperature span of 2 or 5°C was utilized and the midpoint of the range was lowered as the temperature decreased to ensure that the exothermic events associated with freezing occurred within the temperature span selected on the camera. Temperature spans larger than 5°C did not enable resolution of the small exothermic events associated with ice formation and propagation in or on the surface of the plants.

Ice$^+$ Bacteria

The source and characteristics of *P. syringae* strain Cit7 have been described (Lindow, 1985). This Ice$^+$ bacterium was grown for 48 to 96 h on plates of King's medium B (King et al., 1954) at 20°C. Cells were harvested with a sterile toothpick and suspended in sterile, distilled water.

Freezing Studies

Drops of water suspensions of Ice$^+$ bacterial strain Cit7 ranging in size from 0.5–50.0 μL were placed on a thermoelectric cooling plate (Cambion, Midland Ross, Cambridge, MA) set at 5°C to determine the smallest droplet in which ice nucleation could be determined. The temperature of the cooling plate was then decreased to −5.0°C, which was sufficient to cause all of the droplets to freeze.

All plants were cooled in a refrigerated incubator (model 3825, Forma Scientific, Marietta, OH). The lowest temperature obtainable in this incubator was about −5.0°C. Plant material was placed in the incubator with the temperature set either at its lowest limit or at 0°C. In the former case, the temperature of the chamber warmed to about 2°C when the chamber door was opened and the plants were placed within the chamber. When the door was closed there was a rapid decrease in temperature to −1°C, followed by a slow cooling to about −5°C during a period of about 20 min. In the latter case, cooling rates of 1.0 to 2.0°C h^{-1} were utilized. No difference was observed in nucleation temperature or in the pattern of ice propagation between the two cooling rates. In the examples presented, plant materials were shielded from direct exposure to air currents produced by the fan within the incubator by separating the incubator into two compartments with a piece of plywood. Plant materials utilized in this study were potted, greenhouse-grown bean plants (*Phaseolus vulgaris* cv Bush Blue Lake), as well as cut, flowering shoots and vegetative stems of peach (*Prunus persica* cv Loring), cut, flowering shoots of apple (*Malus domestica* cv Rome), and cut, terminal shoots of rhododendron (*Rhododendron* sp. cv Olga), all of which were collected from field plantings at the research station in Kearneysville, WV, between March and April 1995. Plant material was either placed in the incubator dry, misted with water, or inoculated with a 2.0-μL droplet of a suspension of Ice$^+$ bacterial strain Cit7 placed at specific locations on the plant. Experiments were repeated numerous times (in some cases more than 25 individual replicates) and representative images were selected for presentation.

RESULTS

Determination of Accuracy and Sensitivity

The freezing of droplets of a suspension of Ice$^+$ bacteria as small as 0.5 μL on a thermoelectric cooling plate were clearly detectable using the IR radiometer (Fig. 1, seen as a brightening of the drop due to the release of heat of fusion). Bean leaf surface temperatures measured using 30-gauge copper-constantan thermocouples and the IR radiometer were very similar at both slow and fast cooling rates in an advective cooling chamber (Fig. 2). Although air and dry leaf surface temperatures were similar, water droplets on the surface of the leaves were about 1°C cooler than the leaves, presumably due to evaporative cooling (Fig. 2A). These preliminary experiments demonstrated that the IR equipment (a) was capable of detecting freezing of water brought about by the release of the latent heat of fusion, (b) was sensitive enough to detect freezing of small units of water, and (c) could accurately track temperatures of cooling objects.

[2] Mention of a trade name or specific equipment does not constitute a guarantee, warranty, or endorsement of the product.

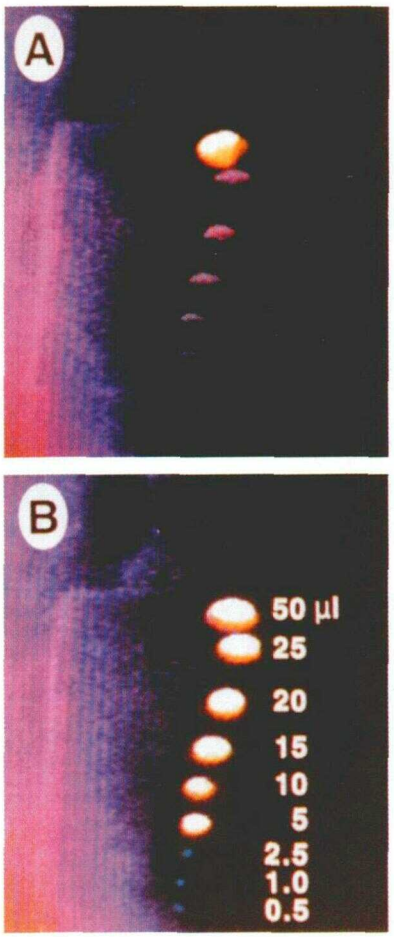

Figure 1. Freezing of droplets of a suspension of *P. syringae* on a thermoelectric cooling plate as viewed with IR video thermography. Droplets ranged in size from 0.5 to 50.0 µL. A, Freezing of the largest droplet is visible as a sudden brightening of the droplet brought about by the release of latent heat. B, Freezing of additional droplets is visible. Although not shown, freezing of droplets as small as 0.5 µL was clearly visible.

Nucleation of a Bean Plant with an Ice$^+$ Bacterium

A 2.0-µL droplet of a suspension of an Ice$^+$ bacterial strain was placed on the left half of a bean leaf and a slightly larger droplet of water was placed on the right side (Fig. 3). The droplet of water appeared black because it was at a lower temperature than the subtending leaf, probably due to evaporative cooling, and was out of the temperature range set for the display device. The display temperature range was 2°C and the chosen color palette rendered areas with temperatures below that range as black and those above as white.

The small droplet of Ice$^+$ bacterial cells was the first to freeze, as detected by the sudden warming of the droplet. Freezing of the leaf was detected 2 min, 25 s later at the site of the bacterial droplet (Fig. 3B). Ice then propagated throughout the entire leaf, down through the petiole, and throughout the rest of the plant, again seen as a sudden warming of the tissue (Fig. 3, C–E). The temperature of the freezing leaf was initially at least 0.3°C warmer than the unfrozen parts of the leaf, allowing clear visualization of the ice/water boundary during ice propagation. The temperature difference between the frozen and unfrozen portion of the leaf continued to increase with time as more water froze and ice accumulated. The drop of water on the surface of the leaf froze independently at least 2.5 min after the subtending portion of the leaf had frozen (Fig. 3F).

When numerous drops of water were placed on the surface of the bean leaf, each drop froze in a random manner over several minutes following the first freezing event, independently of other droplets and the freezing of the subtending portion of the leaf (data not shown). Whenever Ice$^+$ bacteria were placed on the surface of the leaf, with or without additional droplets of water, initial nucleation of the leaf was induced at the site of the placement of Ice$^+$ bacteria. In the absence of introduced Ice$^+$ bacteria, nucleation of the leaf was induced at the site of a droplet of frozen water that had previously nucleated independently. Plants with dry leaf surfaces remained unfrozen to at least −5°C (the lower limit of the incubation chamber used in this study). Under the advective conditions present in our refrigerated incubator, surface temperature differences between plant parts were minimal. At the time initial ice nucleation events occurred, most portions of a leaf, flower, or stem were within 0.5°C of each other, as evidenced by their homogeneous color (Figs. 3–8).

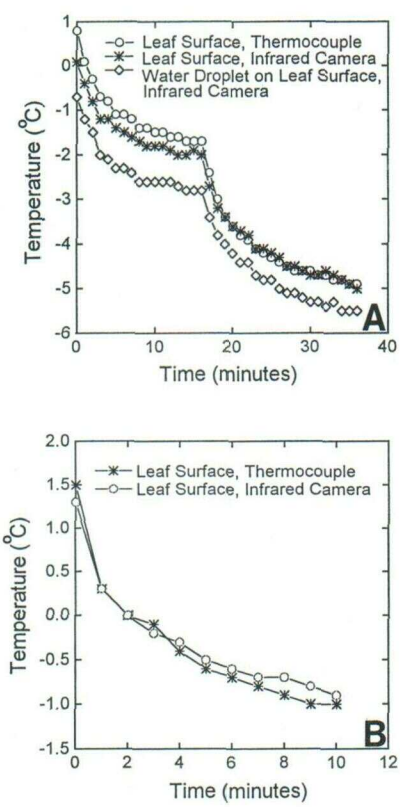

Figure 2. Comparison of bean (cv Blue Lake) leaf temperatures during a cooling experiment as detected using a copper-constantan thermocouple or an IR camera. Temperature of a water droplet undergoing evaporative cooling on the surface of the bean leaf is also plotted (A). Two different cooling regimes are represented in A and B.

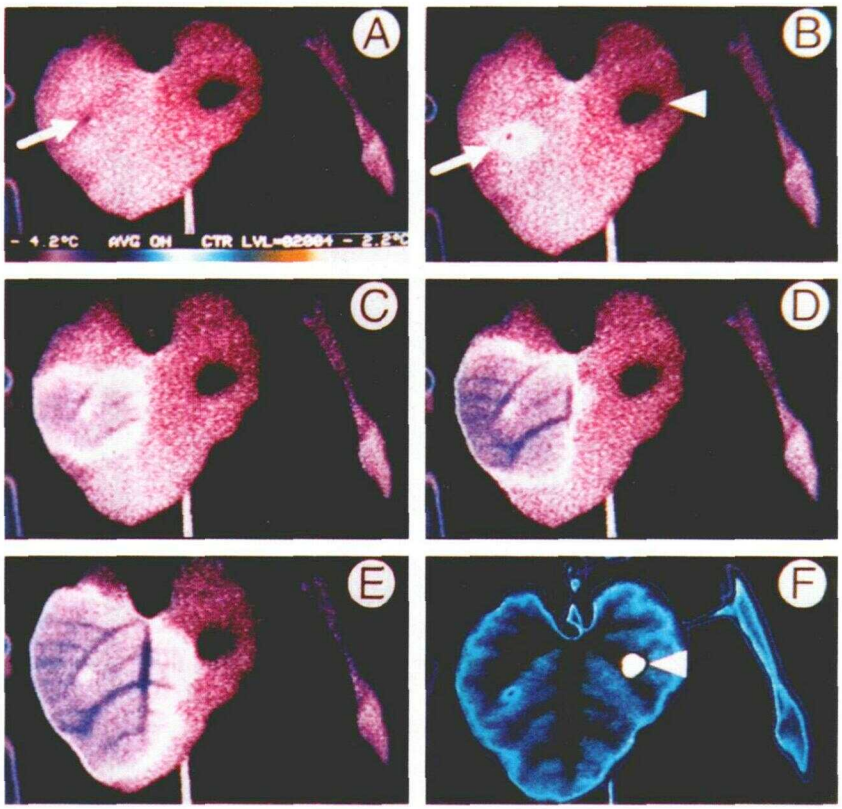

Figure 3. Ice nucleation and ice propagation in a bean (cv Blue Lake) plant in which either a 2.0-μL droplet of a suspension of *P. syringae* (A, arrow) or a drop of deionized water (B, dart) was placed on the surface of the leaf. The drop of water is cooler than the surface of the leaf due to evaporative cooling (seen as a black spot as the actual temperature of the water drop was out of the range set for display of temperatures sensed by the IR imager). The droplet containing Ice^+ bacterial cells was the first to freeze, and that ice then induced ice nucleation in the bean leaf seen as a whitish zone around the Ice^+ bacterial cells. (B). Ice then propagated throughout the leaf and into the rest of the plant (B–F). Freezing of the water droplet on the surface of the leaf occurred over 2 min after most of the water in the subtending leaf had frozen and the exothermic response had dissipated, allowing the leaf to resume cooling (F, blue color).

As illustrated in Figure 4, rates of ice propagation could also be determined using the IR radiometer. When ice nucleation was induced in the stem of the bean plant by the application of Ice^+ bacteria, ice propagation occurred at a slower rate in the nodal area than in the internodal portions of the stem. Once through the internode, ice propagation resumed a rate similar to that in the subtending internode.

Ice Nucleation in Peach Stems and Flowers

When Ice^+ bacteria were applied to the base of the gynoecium in peach flowers, the bacteria were the first to freeze, as indicated by the bright region at the base of the treated flower (Fig. 5A), and subsequently induced nucleation in the flower (Fig. 5B). Initial freezing of bacteria

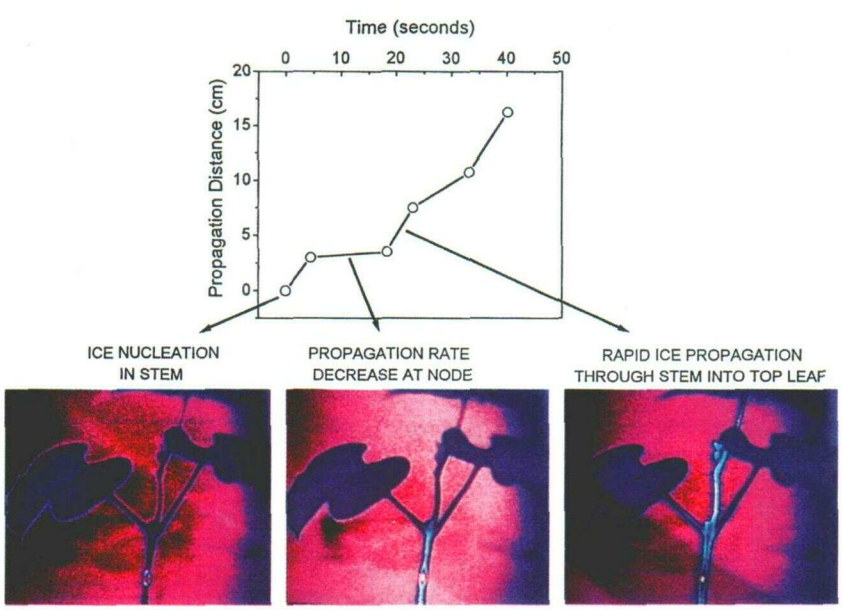

Figure 4. Determination of rates of ice propagation in a bean plant using IR video thermography. The initial site of ice nucleation occurred in the stem at the site of the placement of a drop of culture of *P. syringae* (left image, white spot near center of stem). Propagation of ice from the site of nucleation up through the stem into the terminal leaf is visible as a light blue-white band increasing in length with time (center and right image). The length of the ice front in the stem as a function of time is plotted in the graph above the figures.

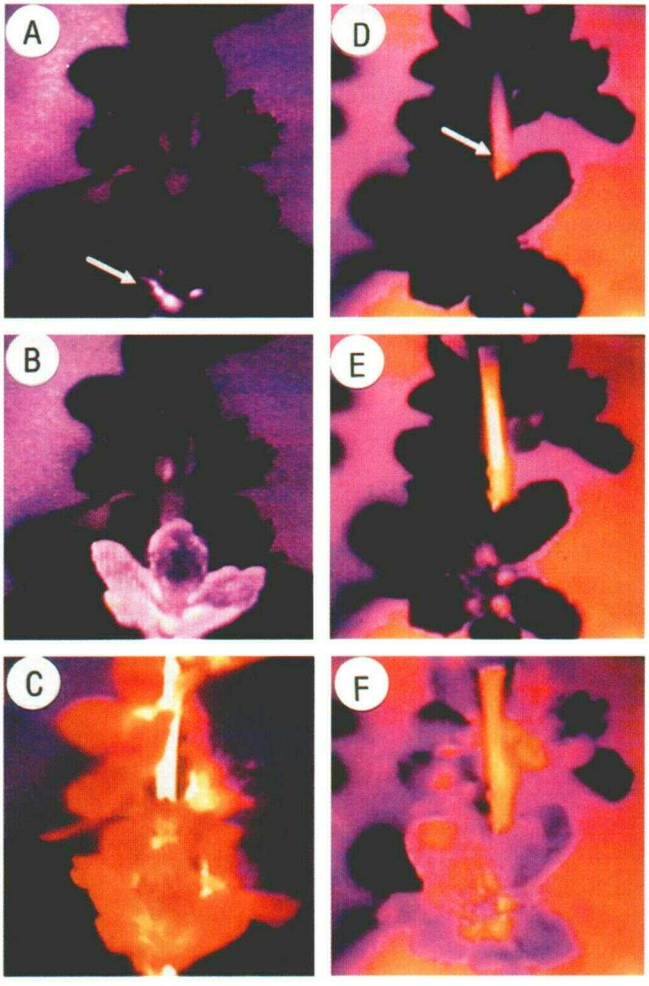

Figure 5. Ice nucleation and propagation in flowering shoots of peach (cv Loring) as seen with IR video thermography. A to C, Shoot with a droplet of suspension of Ice^+ bacterial cells (*P. syringae*) placed at the base of the gynoecium of a flower. The drop of Ice^+ bacteria was the first to freeze (A, light colored spot at arrow) and then induced ice nucleation of the flower (B). Ice then propagated into the stem and out to other flowers along the shoot (B and C). D to F, Freezing of peach shoot in the absence of introduced Ice^+ bacteria. Ice nucleation initially occurred in the stem (D, arrow) and then propagated along the shoot and out to the flowers (E and F).

occurred at about −3.0°C. Ice then propagated into the stem and into the other open flowers (Fig. 5, B and C; note brightening of tissue as ice propagates into the tissue). In the absence of applied Ice^+ bacteria, initial ice nucleation was observed primarily in stem tissues (Fig. 5D) at temperatures in the range of −3.0 to −4.2°C. Subsequently, ice propagated along the stem and into the attached flowers (Fig. 5, E and F). These data indicate that ice can readily propagate along a shoot and to and from attached flowers. Additionally, a single nucleation event can trigger freezing of an entire shoot.

Ice nucleation within the peach stem initially occurred in cortical tissues and then propagated into xylem and pith tissues (Fig. 6, A–C). The rate of vertical propagation in cortical tissues was faster than propagation circumferentially (around the stem). This was evidenced by the time elapsed before freezing was visible on the right side of the shoot compared with the time elapsed for freezing to be visible a considerable distance down from the initial site of ice formation in the left side of the shoot. In some peach twigs, the freezing process was more complex. Examples of flowers along a stem harboring only indigenous ice nuclei frozen independently of each other and prior to the freezing of the stem tissue were noted (data not shown). Additionally, the presence of both frozen and unfrozen flower buds attached to a frozen stem were also observed. In experiments in which thermocouples were attached to shoots, ice nucleation often occurred at the site of the thermocouple.

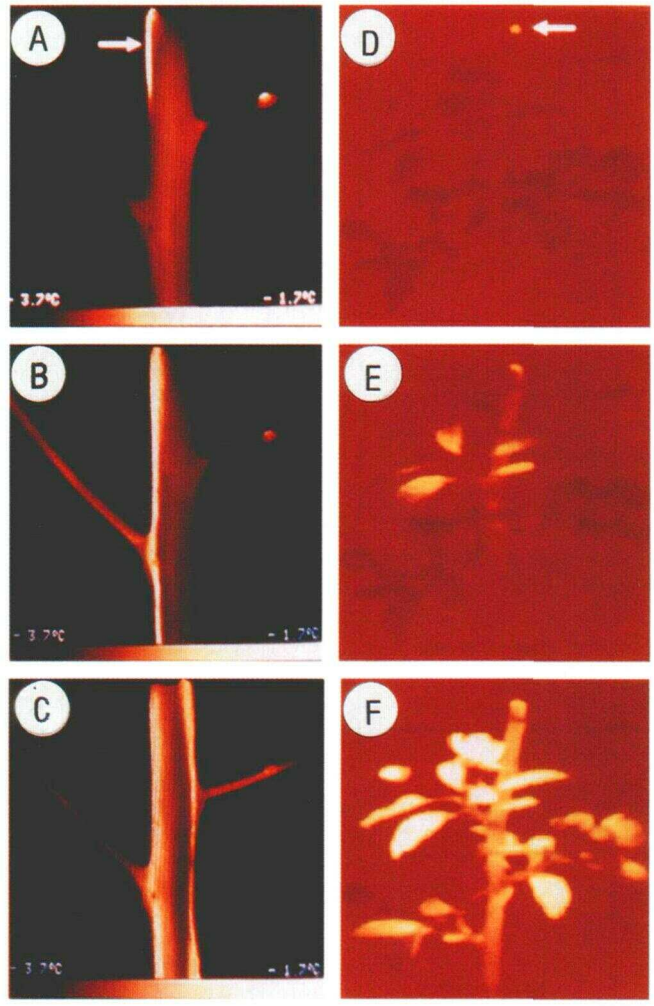

Figure 6. Ice nucleation and propagation in a peach (cv Loring) shoot that was split in half longitudinally (A–C) and in a detached flowering shoot of apple (cv Rome) in the absence of introduced Ice^+ bacteria (D–F), as seen with IR video thermography. Ice nucleation occurred in the cortical tissue (A, arrow, visible as a light area) and propagated through the cortical tissues both vertically and circumferentially (although at a greater rate vertically) and then into xylem and pith tissues (B and C). Ice propagation from the main branch into smaller branches is also visible (B and C). Ice nucleation occurred at the cut surface of the flowering apple shoot (D, arrow, light area) and then propagated down through the shoot and out into the leaves and clusters of flowers (E and F).

Ice Nucleation in Apple Flower Clusters

In the absence of applied Ice^+ bacteria, initial ice nucleation occurred in apple stem tissues (Fig. 6D, bright area at the top) and ice propagated into flower clusters (Fig. 6, E and F). When droplets (approximately 2.0 μL) of an Ice^+ bacterial suspension were placed either on leaves or directly on the flower clusters, these droplets froze first and served as the site of nucleation for the apple stem and flower clusters (Fig. 7). Unfrozen droplets were cooler (appearing black) than the surrounding tissue due to evaporative cooling (Fig. 7A). As droplets froze, an abrupt change in temperature was detected (Fig. 7, bright spots).

Ice Nucleation in Rhododendron Shoots

In contrast to the other woody plants examined, the site of initial ice nucleation in rhododendron was within the stem of the plant regardless of whether Ice^+ bacteria were applied to the leaf surface of this plant (Fig. 8). Even in cases in which a droplet of Ice^+ bacteria had frozen on the surface of the leaf, the frozen droplet did not induce freezing of the underlying leaf. The terminal cluster of buds (imaged as blue in the center of each figure) cooled at a slower rate than the leaf material, i.e. at any given time during the course of temperature observations (up until the time the leaves froze), the bud tissues were warmer than the surrounding leaves. This perhaps indicates that the buds contained more water than leaves and that the water acted as a thermal reservoir. Alternatively, the terminal buds may have a higher metabolic rate.

DISCUSSION

The data from this initial study indicate that IR video-imaging systems permit the freezing process in plants to be directly observed. The temperature and spatial resolution of the IR-imaging radiometer enabled us to clearly define the initial site of ice nucleation, as well as to monitor the ice front as it spread into the surrounding tissues. The IR camera was capable of detecting the freezing of droplets as small as 0.5 μL (Fig. 1) and estimated the surface temperatures of plant tissue as accurately as a copper-constantan thermocouple (Fig. 2).

A unique aspect of this technology is that one can determine the initial sites of ice nucleation and the temperature at the time of nucleation, as well as monitor the subsequent propagation of ice throughout the plant. Whereas there are other methods for detecting and quantifying the number of ice nuclei in plant tissue and detecting ice formation itself, thermal IR thermography provides unique information not available from these other techniques. Ice nuclei that are active at the warmest temperature are of primary importance because they presumably initiate ice that subsequently propagates throughout the plant. Thermoelectric devices such as copper-constantan thermocouples (Quamme et al., 1972; Ashworth and Davis, 1984; Anderson and Ashworth, 1985; Ashworth et al., 1985; Andrews et al., 1986; Quamme, 1995) can provide an estimate of the temperature at which the warmest ice nuclei catalyzed ice. Unfortunately, it is not possible to determine either the site or the number of ice nucleation events using this technology; detecting the exothermic response of plants at a given point merely indicates the temperature of that part of the plant when ice formation occurred at that site. One has to assume that the temperature was similar at the site where ice nucleation occurred.

Arrays of thermocouples have been used to refine the estimates of where ice formation occurred and the rate of ice propagation (Single, 1964; Single and Olien, 1967; Ashworth et al., 1985; Yelenosky, 1991a, 1991b). Although multiple thermocouples are an improvement over the use of a single temperature probe for assessing ice formation in a plant, the accuracy with which conclusions on the site of ice formation and the rate of ice propagation can be made are proportional to the spatial density of the temperature probes. The practicality of this approach decreases rapidly with the number of probes that must be used.

In addition to the use of an array of thermocouples to monitor ice propagation, cryomicroscopy has also been

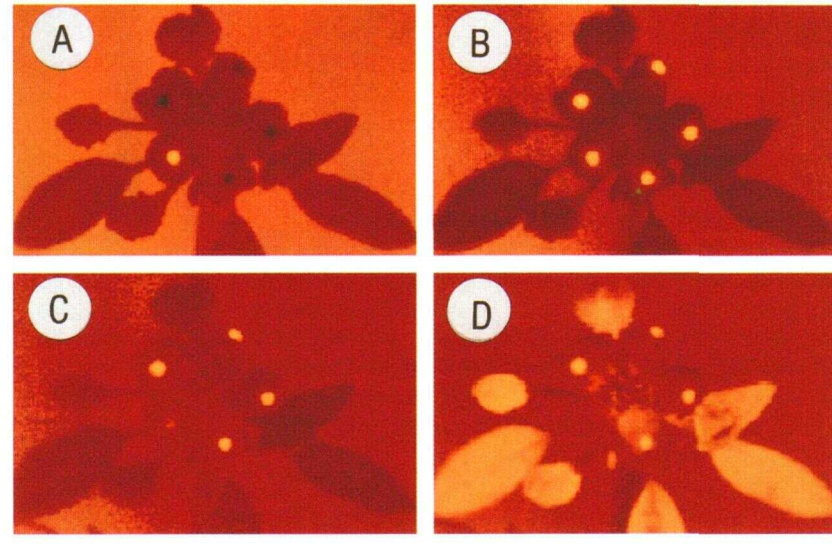

Figure 7. Ice nucleation and propagation in a detached apple flower cluster with a single 2.0-μL drop of a suspension of an Ice^+ strain of *P. syringae* placed on each petal of a large flower, as seen with IR video thermography. A, Evaporative cooling of several droplets to a temperature below that of the petal is visible as dark spots. One droplet nucleated and was warmer than the petals and other droplets (yellow spot). B, Freezing of the remaining drops occurred by the time of capture of the image. C and D, This induced freezing of the flower, and ice then propagated into the rest of the cluster (yellow).

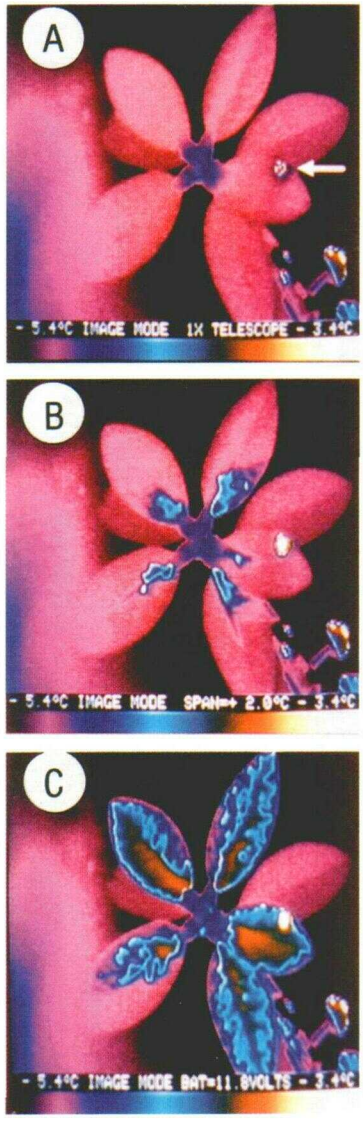

Figure 8. Ice nucleation and propagation in a detached, terminal portion of a shoot of rhododendron (cv Olga), as seen with IR video thermography. Despite the freezing of a drop of a suspension of *P. syringae* that had been placed on a leaf (A, arrow), initial nucleation of the shoot occurred in the subtending stem and then propagated into leaves and buds, causing a warming of the tissue (B and C, blue- and rust-colored areas).

used (Kaku, 1964; Cary and Mayland, 1970; Burke et al., 1976). In the former approach, a large number of temperature sensors would be needed to assess accurately the rate of propagation over substantial distances or through successive tissue types. The latter approach is limited to small distances because of the limitations of the field of view of the microscope used and cannot be used to study freezing under field conditions. Thermal IR imaging seems ideally suited for determining both the number and sites of ice nucleation events, as well as the subsequent rate of ice propagation. For example, differences in the rate of ice propagation through nodal and internodal regions of bean were easily detected and measured using this technique (Fig. 4) and confirmed previous observations that differences in xylem anatomy at nodes slow the rate of ice propagation (Zámećník et al., 1994). Likewise, it is possible to determine the temperature at the site and the time of ice nucleation using IR-imaging techniques. Although little temperature difference was observed within plants frozen in the advective conditions used here, others have noted that substantial differences in temperature occur within a given plant under radiative freezing conditions in the field (Modlibowska, 1962; Renquist, 1985). IR imaging should prove particularly useful for determining ice nucleation temperatures of plants under field conditions.

The ability of Ice^+ bacteria to induce freezing in a variety of plant species was clearly observed in the present study (Figs. 3–8), as was the indication of putative, intrinsic agents in woody plants (Figs. 5, D–F, 6, and 8). It is interesting that cortical tissues appeared to be more efficient at ice nucleation than xylem tissues (Figs. 6, A–C). In peach stems, ice propagated vertically within the cortical tissues for a considerable distance prior to the complete freezing of the xylem tissues. We also observed that the site of placement of a thermocouple was often the site of initial freezing (data not shown). Why thermocouples should induce ice nucleation is not known; however, thermocouples may represent a significant thermal mass and conduct heat away from the tissue. Alternatively, Vali (1995) indicated that the orienting effects of electric fields are believed to play a role in ice nucleation, although this has been difficult to demonstrate. The pattern of ice nucleation in peach stems and the induction of freezing at the site of Ice^+ bacterial cells and by thermocouples are examples of information not obtainable with prior technology.

A comparison of sequential images of bean leaves (Fig. 3) and rhododendron leaves (Fig. 8) undergoing nucleation and freezing indicated that the freezing of the rhododendron leaves was independent of the freezing of the Ice^+ bacterial cells, whereas the nucleation of the bean plant was directly induced by the prior freezing of droplets containing bacterial cells. In the example shown, nucleation of water droplets on the bean leaf occurred at least 2 min after the leaf had frozen. The pattern of ice formation (as evidenced by its exothermic fingerprint) appeared more dendritic in rhododendron than in bean leaves, where it appeared to traverse the leaves in a wave-like pattern. Again, these details of ice nucleation and propagation were not previously discernible and may reflect differences in tissue water content, the effect of antifreeze proteins or sugars on ice growth, or other evolutionary adaptations developed by plants to deal with the adverse effects of ice formation in their tissues. These findings add complexity to our view of inoculation of plants by ice.

The results of the present study indicate that ice nucleation and propagation in plants can be quite complex. We observed freezing of plants that was clearly induced by applied Ice^+ bacteria. Ice nucleation by putative intrinsic nucleators, despite the presence of applied Ice^+ bacteria, was also observed. We did not assay for the presence of Ice^+ bacteria on the stem segments that were frozen in this study. While viable Ice^+ bacteria may have been present on parts of the plant not inoculated with these species, the

population sizes on the woody parts of deciduous tree fruits are commonly reported to be low (Andrews et al., 1986; Gross et al., 1988). In some cases, open flower buds were the primary sites of ice nucleation in peach shoots, whereas in other samples ice nucleation occurred in stem tissues.

Overall, the use of current IR video technology is an excellent tool for elucidating the process of freezing and ice propagation in plants. Having demonstrated that ice nucleation and propagation can be readily observed in laboratory studies using IR imaging, we are now in the process of documenting the freezing of various plant species under a variety of field conditions. We hope that these studies will result in a more comprehensive knowledge of ice formation in plants and serve as the basis for developing and refining frost protection technologies.

Received July 8, 1996; accepted October 31, 1996.

LITERATURE CITED

Anderson JA, Ashworth EN (1985) Ice nucleation in tomato plants. J Am Soc Hortic Sci **110:** 291–296

Andrews PK, Proebsting EL, Gross DC (1986) Ice nucleation and supercooling in freeze-sensitive peach and sweet cherry tissues. J Am Soc Hortic Sci **111:** 232–236

Ashworth EN, Anderson JA, Davis GA, Lightner GW (1985) Ice formation in *Prunus persica* under field conditions. J Am Soc Hortic Sci **110:** 322–324

Ashworth EN, Davis GA (1984) Ice nucleation within peach trees. J Am Soc Hortic Sci **109:** 198–201

Ashworth EN, Kieft TL (1995) Ice nucleation activity associated with plants and fungi. *In* RE Lee, GJ Warren, LV Gusta, eds, Biological Ice Nucleation and Its Applications. American Phytopathological Society, St. Paul, MN, pp 137–162

Burke MJ, Gusta LA, Quamme HA, Weiser CJ, Li PH (1976) Freezing and injury to plants. Annu Rev Plant Physiol **27:** 507–528

Cary JW, Mayland HF (1970) Factors influencing freezing of supercooled water in tender plants. Agron J **62:** 715–719

Ceccardi TL, Heath RL, Ting IP (1995) Low-temperature exotherm measurement using infrared thermography. Hortscience **30:** 140–142

Gross DC, Proebsting EL Jr, Andrews PK (1984) The effects of ice nucleation-active bacteria on the temperatures of ice nucleation and low temperature susceptibilities of *Prunus* flower buds at various stages of development. J Am Soc Hortic Sci **109:** 375–380

Gross DC, Proebsting EL Jr, MacCrindle-Zimmerman H (1988) Development, distribution, and characteristics of intrinsic, nonbacterial ice nuclei in *Prunus* wood. Plant Physiol **88:** 915–922.

Hirano SS, Baker LS, Upper CD (1985) Ice nucleation temperature of individual leaves in relation to population sizes of ice nucleation active bacteria and frost injury. Plant Physiol **77:** 259–265

Kaku S (1964) Undercooling points and frost resistance in mature and immature leaf tissues of some evergreen plants. Bot Mag **77:** 283–289

King EO, Ward MK, Raney DE (1954) Two simple media for the demonstration of pyocyanin and fluorescein. J Lab Clin Med **44:** 301–307

Le Grice P, Fuller MP, Campbell A (1993) An investigation of the potential use of thermal imaging technology in the study of frost damage to sensitive crops. *In* Proceedings of the International Conference on Biological Ice Nucleation, Ed 6, University of Wyoming, Laramie, p 4

Lindow SE (1982) Population dynamics of epiphytic ice nucleation active bacteria on frost sensitive plants and frost control by means of antagonistic bacteria. *In* PH Li, A Sakai, eds, Plant Cold Hardiness. Academic Press, New York, pp 395–416

Lindow SE (1983) The role of bacterial ice nucleation in frost injury to plants. Annu Rev Phytopathol **21:** 363–384

Lindow SE (1985) Integrated control and role of antibiosis in biological control of fireblight and frost injury. *In* C Windels, SE Lindow, eds, Biological Control on the Phylloplane. American Phytopathological Society, Minneapolis, MN, pp 83–115

Lindow SE (1995) Control of epiphytic ice nucleation-active bacteria for management of plant frost injury. *In* RE Lee, GJ Warren, LV Gusta, eds, Biological Ice Nucleation and Its Applications. American Phytopathological Society, St. Paul, MN, pp 239–256

Lindow SE, Arny DC, Barchet WR, Upper CD (1978) The role of bacterial ice nuclei in frost injury to sensitive plants. *In* PH Li, ed, Plant Cold Hardiness and Freezing Stress. Academic Press, New York, pp 249–263

Lindow SE, Arny DC, Upper CD (1982) Bacterial ice nucleation. A factor in frost injury to plants. Plant Physiol **70:** 1084–1089

Lindow SE, Arny DC, Upper CD (1983) Biological control of frost injury. II. Establishment and effects of an antagonistic *Erwinia herbicola* isolate on corn in the field. Phytopathology **73:** 1102–1106

Lucas JW (1954) Subcooling and ice nucleation in lemon. Plant Physiol **29:** 245

Marcellos HW, Single WV (1976) Ice nucleation on wheat. Agric Meteorol **16:** 125–119

Marcellos HW, Single WV (1979) Supercooling and heterogeneous nucleation of freezing in tissues of tender plants. Cryobiology **16:** 74–77

Modlibowska I (1962) Some factors affecting supercooling of fruit blossoms. J Hortic Sci **37:** 249–261

Proebsting EL Jr, Andrews PK, Gross D (1982) Supercooling young developing fruit and floral buds in deciduous orchards. Hortic Sci **17:** 67–68

Proebsting EL Jr, Gross DC (1988) Field evaluations of frost injury to deciduous fruit trees as influenced by ice-nucleation-active *Pseudomonas syringae*. J Am Soc Hortic Sci **113:** 498–506

Quamme HA (1995) Deep supercooling in buds of woody plants. *In* RE Lee, GJ Warren, LV Gusta, eds, Biological Ice Nucleation and Its Applications. American Phytopathological Society, St. Paul, MN, pp 183–200

Quamme HA, Stushnoff C, Weiser CJ (1972) The relationship of exotherms to cold injury in apple stem tissues. J Am Soc Hortic Sci **97:** 608–613

Renquist AR (1985) The extent of fruit bud radiant cooling in relation to freeze protection with fans. Agric For Meteorol **36:** 1–6

Single WV (1964) Studies on frost injury to wheat. II. Ice formation within the plant. Aust J Agric Res **15:** 869–875

Single WV, Olien CR (1967) Freezing processes in wheat stems. Aust J Biol Sci **20:** 1025–1028

Vali G (1995) Principles of ice nucleation. *In* RE Lee, GJ Warren, LV Gusta, eds, Biological Ice Nucleation and Its Applications. American Phytopathological Society, St. Paul, MN, pp 1–28

Yelenosky G (1991a) Supercooling and freezing in the main stem of Valencia orange trees. Cryobiology **28:** 382–390

Yelenosky G (1991b) Apparent nucleation and freezing in different parts of young citrus trees during controlled freezes. Hortscience **26:** 576–579

Zámečník J, Bielová J, Grospietsch M (1994) Safety zone as a barrier to root-shoot ice propagation. Plant Soil Sci **167:** 149–155